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**Studies on the mode of action of intra-amniotically
and extra-amniotically injected hypertonic saline
in therapeutic abortion**

by Björn Gustavii

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STUDIES ON THE MODE OF ACTION
OF INTRA-AMNIOTICALLY AND
EXTRA-AMNIOTICALLY INJECTED
HYPERTONIC SALINE IN
THERAPEUTIC ABORTION

BY
BJÖRN GUSTAVII

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- II Gustavii, B.: Intra-amniotic and extra-amniotic injection of sodium chloride solution: Amniotic fluid salinity and abortifacient effect. *Amer J Obstet Gynec*. In press.
- III Gustavii, B. & Brunk, U.: A histological study of the effect on the placenta of intra-amniotically and extra-amniotically injected hypertonic saline in therapeutic abortion. *Acta Obstet Gynec Scand* 51: 121, 1972.
- IV Brunk, U. & Gustavii, B.: Lability of human decidual cells. In vitro effects of autolysis and osmotic stress. *Amer J Obstet Gynec* 115: 811, 1973.
- V Gustavii, B. & Brunk, U.: Lability of human decidual cells. In vivo effects of hypertonic saline. *Acta Obstet Gynec Scand*. In press.
- VI Gustavii, B. & Gréen, K.: Release of prostaglandin $F_{2\alpha}$ following injection of hypertonic saline for therapeutic abortion: A preliminary study. *Amer J Obstet Gynec* 114: 1099, 1972.

References to these papers will be made by the Roman numerals given above.

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INTRODUCTION

Despite much research the mechanism controlling the onset of labor remains unknown. A method commonly used to attack the problem is to study women undergoing therapeutic abortion in midpregnancy. It may be argued, of course, that the induction of abortion in midpregnancy has little in common with the spontaneous onset of labor at term. The processes are similar, however, in that they involve a sudden increase in the strength and frequency of uterine contractions, leading to cervical dilatation and expulsion of the product of conception.

Many substances, chemically and physically unrelated, are used for terminating mid-trimester pregnancies, e.g. hypertonic saline, hypertonic glucose, 40 % formalin (for ref. see Bengtsson 1968) and 0.1 % rivanol (Inge-manson 1973), as well as prostaglandins (Wiqvist et al. 1972). Of these substances, hypertonic saline is the one most widely used.

Hypertonic saline used for therapeutic abortion can be injected either between the fetal membranes and the uterine wall (extra-amniotic injection) or into the amniotic sac (intra-amniotic injection). The two methods differ not only in the route of injection, but also in that the intra-amniotic method involves previous aspiration of the amniotic fluid. In spite of these differences, the interval between injection and abortion is approximately the same with both methods (~ 36 hours) when a 20 % saline solution is used (Bengtsson & Ryde 1967, Jansson et al. 1967).

Several hypotheses have been produced to explain the mechanism by which hypertonic

saline and other solutions induce abortion, and the more important of these are reviewed below.

Progesterone withdrawal

Bengtsson & Csapo (1962) have suggested the possibility that intra-amniotically injected hypertonic saline, by virtue of its effect on the placenta, eliminates a protective mechanism normally blocking myometrial activity. They assumed that this block is maintained by the effect of progesterone on the myometrium. The hypothesis was based mainly on the following observations in hypertonic saline-abortions induced by the intra-amniotic method:

- (1) Necrotizing aseptic placentitis (Bengtsson & Stormby 1962).
- (2) Fall in progesterone concentration in peripheral venous blood (Luukkainen cit. by Csapo 1961).
- (3) Decrease in urinary excretion of pregnandiol (Bengtsson & Stormby 1962).
- (4) Significant prolongation of the interval between injection and abortion by oral treatment with gestagens (Bengtsson & Csapo 1962).

This hypothesis has been debated. Although subchorionic necrosis of villi has been found by various investigators (Strauss 1963, Weingold et al. 1965, Wynn 1965, Christie et al. 1966, Gochberg & Reid 1966, Pathak 1968, Jakobovits et al. 1970), the changes are believed to be far too limited to have any definite effect on the placental hormone production (Wynn 1965, Christie et al. 1966). Some workers (Wiest et al. 1966, Wiest 1967,

Csapo et al. 1969, Csapo et al. 1970, Wiest et al. 1970, Holmdahl et al. 1971) have confirmed the decrease in peripheral plasma progesterone, though Holmdahl et al. (1971) could not find any correlation between this decrease and the onset of abortion. Several workers (Fuchs et al. 1965, Short et al. 1965, Kerr et al. 1966, Osborn et al. 1968) have also failed to demonstrate a fall in plasma progesterone of peripheral venous blood or uterine venous blood before development of labor-like contractions. As to the effect of gestagens, some claim that they delay abortion (Gennser et al. 1968, Ruttner 1969), while others have failed to demonstrate such an effect (Møller et al. 1964). Hence, investigations of the progesterone withdrawal hypothesis have given conflicting results.

Increased uterine volume

The significance of uterine volume increase as a factor in the initiation of myometrial activity has been stressed by some authors (Csapo et al. 1963). Using inulin dilution technique, the amniotic fluid volume during the first 3 hours following intra-amniotic injection of hypertonic saline has been calculated to increase by approximately 25–30 % (Wagner 1966). However, it has not been demonstrated that the amniotic fluid volume is increased at the time of effective uterine activity. Moreover, in normal midpregnancy, it is not possible to induce abortion even by extensive increase in the volume by injection of isotonic saline into the amniotic sac (Pulkkinen & Kivikovski 1969). Hence, the volume increase can hardly be a decisive factor in the initiation of hypertonic saline-abortion.

Fetal death

By continuously recording the activity of the fetal heart after intra-amniotic injection of hypertonic saline, the interval between the injection and the death of the fetus has been found to be 3–5 hours (Kovács et al. 1970).

In hypertonic saline abortion, the fetus is rarely delivered alive (King et al. 1964, Kovács et al. 1970). However, in a study of abortions induced in the late second trimester by 0.1 % rivanol solution injected extra-amniotically, all the fetuses were alive at delivery (Manabe 1969). The death of the fetus can therefore not be taken as a prerequisite for the onset of abortion.

Oxytocin release

It has been shown that intravenous infusion of hypertonic saline stimulates uterine motility (Hendricks et al. 1959) and that prolonged intravenous infusion of concentrated oxytocin solution induces abortion in late midpregnancy (Burnhill et al. 1962). Based on these observations, it has been suggested that intra-amniotic hypertonic saline stimulates uterine contractions by releasing neurohypophyseal hormones (Wiqvist & Eriksson 1964). The patient's intense thirst during the first day after the injection support this view (Wiqvist & Eriksson 1964). The uterine distension caused by the increased volume of amniotic fluid following injection of hypertonic saline might be another stimulus of the release of neurohypophyseal hormones by activating a neurohumoral reflex (Berkowitz & Fuchs 1971). However, since the uterine contractions develop slowly, it seems unlikely that large amounts of oxytocin are released (Wagner 1966). Moreover, at the time of effective uterine activity, the sodium concentration (Weingold et al. 1965, Csapo 1966, Andersson & Turnbull 1968, Pathak 1968, Wong et al. 1972) and the osmolality (Andersson & Turnbull 1968) of the serum have already returned to their initial levels. In addition, the abortifacient effect of 0.1 % rivanol is equal to that of hypertonic saline (Ingemanson 1973), in spite of the fact that 0.1 % rivanol is *hypotonic* and consequently *decreases* the serum osmolality. It is thus unlikely that the initial increase in the blood osmotic pressure

following intrauterine injection of hypertonic saline is responsible for the initiation of abortion.

Effect on the decidua

Most reports of histological examination of the placenta after intrauterine injection of hypertonic saline do not mention the decidua (Bengtsson & Stormby 1962, Oram et al. 1963, Sciarra et al. 1964, Weingold et al. 1965, Wynn 1965, Christie et al. 1966, Gochberg & Reid 1966, Pathak 1968, Jakobovits et al. 1970, Blaustein & Shenker 1971). Jaffin et al. (1962) found "no microscopic evidence of ... deciduitis". Strauss (1963) has briefly commented that "the decidua shows occasional necrosis and haemorrhage at the edge of the placenta". Since the present investigation indicates that the decidua may play a central role in the mode of action of hypertonic saline, a few details about this tissue will be given.

Early in pregnancy the endometrial stroma cells are transformed to large, rounded, or polyhedral elements, known as decidual cells. Their functional significance is not understood.

Mossman (1937) claimed that the basic function of the decidua is to nourish the trophoblast. However, on the basis of histochemical and electron microscopic investigations it has been suggested that, owing to its complex fine structure and glucoprotein inclusions, the decidua may be an endocrinologically active tissue (Wynn 1967).

It is apparent from this review that opinions differ on the mechanism by which hypertonic saline induces abortion. The main question seems to be: Which tissue is the target for the action of the hypertonic saline? Is it an extra-uterine organ (such as the pituitary), the uterus itself, or part of the product of conception? In the present investigation, this problem was first attacked by a study on the effect of hypertonic saline, injected extra-amniotically or intra-amniotically, on the uterine tissues, the placental tissue, and the amniotic fluid. Later, when some results focused the interest on the decidua, this tissue was especially studied histologically and cytochemically in an attempt to elucidate the triggering mechanism of abortion.

MATERIAL AND METHODS

Clinical material

The clinical material consisted of physically healthy women admitted to the department for therapeutic abortion. They were informed of the nature of the trial and consented to participate.

Injection technique

In the first investigations (papers I—III), a Nelaton catheter was used for the extra-amniotic injections. Later, a Foley catheter was found to be more suitable because it reduces the risk of accidental intravascular injection of the hypertonic saline and prevents leakage of saline into the vagina (Gustavii 1972).

Extra-amniotic NELATON catheter-method. This technique (Svane 1960) was used in women in the fifteenth to twentieth week of pregnancy (papers I—III). A No. 12 Nelaton catheter was passed through the cervix and advanced between the uterine wall and the fetal membranes. 150—200 ml of a 20 % saline solution, i.e. 10 ml for each week of pregnancy, was injected through the catheter.

Extra-amniotic FOLEY catheter-method. This technique was used in women in the eleventh to twelfth week of pregnancy (paper V) and fifteenth to twenty-second week of pregnancy (papers V and VI). A No. 12 Foley catheter was placed with its balloon just inside the internal cervical orifice. The balloon was filled with 10 ml fluid. A 20 % saline solution was then injected through the catheter; 50—100 ml in the eleventh to twelfth week of pregnancy, and 150—200 ml in the fifteenth to twenty-

second week of pregnancy. After the injection the catheter was left in position for up to two hours to prevent leakage of the saline into the vagina.

Intra-amniotic method. This technique (Bengtsson 1968) was used in women in the fifteenth to twentieth week of pregnancy. The midline of the abdomen halfway between the fundus uteri and the symphysis was infiltrated with a local anaesthetic. A needle 15 cm long and 3 mm in external diameter was introduced through the anaesthetized area, at right angles to the anterior wall of the uterus. When clear amniotic fluid was flowing freely, a polyethylene catheter was inserted through the needle and advanced 10—15 cm into the amniotic cavity. The needle was thereafter withdrawn. As much amniotic fluid as possible was aspirated through the catheter, and 150—200 ml of a 20 % saline solution was injected into the amniotic sac through the same catheter. In one investigation (paper II) a 5 % saline solution was used instead of the 20 % solution.

Collection of amniotic fluid

Samples of amniotic fluid were obtained either through the polyethylene catheter inserted into the amniotic sac (paper II) or by a trans-abdominal amniocentesis (paper VI).

Analyses

After centrifugation and filtration of the amniotic fluid, its sodium concentration was measured by flame spectrophotometry (Eppendorf). The normal concentration in the eighth

to twenty-fourth week of pregnancy is 128—143 mEq/l (Sinha & Carlton 1970).

Prostaglandin $F_{2\alpha}$ in amniotic fluid was determined by gas chromatography-mass spectrometry with the deuterium carrier technique (Axen et al. 1971). These determinations were performed by Dr. Krister Gréen at the Department of Medical Chemistry, Royal Veterinary College, Stockholm.

The osmolality of the various solutions was measured by freezing-point depression, using a Knauer Halb Micro-osmometer, with a 400 mOsm NaCl solution as a reference.

Radiography

The hypertonic saline was made radiographically visible by the addition of contrast medium (*Isopaque Cerebral*, Nyco A/S, Norway, 15 ml per 100 ml 20 % saline). The solution was injected extra-amniotically through a Nelaton catheter in a volume varying with the length of pregnancy, viz. 11.5 ml (equivalent to 10 ml of 20 % saline) for each week of pregnancy. The first film was exposed after injection of 40 ml of the predetermined volume (test dose); a second one, after injection of the rest of the dose, and then every hour for two to four hours. All radiographs were taken with the patient supine.

Tissue specimens

Placenta and myometrium. Following intra-amniotic or extra-amniotic injection of 20 % saline solution, placentae were obtained either at abortion or by subtotal hysterectomy. (The patients who underwent subtotal hysterectomy had been admitted for both abortion and sterilization).

Fixation was performed in 10 % formaldehyde according to the routine method for surgical specimens. Four blocks of tissue were selected from the central part of the placenta for histological examination. In cases following hysterectomy, parts of the placenta with adjacent myometrium, were processed *en bloc*.

The specimens were embedded in the conventional way in paraffin, cut, and stained with haematoxylin-eosin, PAS and by van Gieson's method. In some cases staining for fibrin was performed with Weigert's technique.

Parietal decidua and free chorionic villi. In two of the histological investigations the examinations were confined to the decidua parietalis (papers IV and V) and the free chorionic villi (paper IV). No attempt was made to evaluate changes in the decidua basalis and trophoblastic shell due to the normally occurring regressive changes in the decidual and trophoblastic cells adjacent to Nitabuch's membrane.

In the *in vitro*-study (paper IV) the parietal decidua and the free chorionic villi were obtained by vacuum aspiration in the ninth to twelfth week of pregnancy. The aspirated material, collected in an empty glass bottle cooled to 0° C by melting ice, was divided into two parts; one was exposed to fixatives of different total and effective osmotic pressure, and the other was allowed to undergo autolysis *in vitro*.

The fixatives used consisted of 3 % formaldehyde (obtained from paraformaldehyde) in Na-cacodylate-HCl buffer (pH 7.4). The concentration of the buffer was either 0.01 M, giving low ("improper") effective osmotic pressure (total osmolality of the fixative solution ~ 1,085 mOsm), or 0.15 M, giving high ("proper") effective osmotic pressure (total osmolality of the fixative solution ~ 1,350 mOsm) (Brunk & Ericsson 1972). The fixation was performed at 0° C for 12 to 18 hours.

The *effective* osmotic pressure might be defined as the osmotic pressure produced by those "particles" which do not pass through the semipermeable cellular membranes. The membranes are not permeable to the buffer ions, which thus constitute the compound in the fixative solution that builds up the effective osmotic pressure. Formaldehyde passes through the membranes fairly freely and accordingly does not contribute much to the effective osmotic

pressure. The total osmotic pressure of a fixative solutions seems to be of minor importance, compared with its effective osmotic pressure. (Brunk & Ericsson 1972.)

The tissues to undergo autolysis *in vitro* were kept at room temperature ($\sim 20^{\circ}\text{C}$) in a humidified container. Every hour up to 12 hours after the initiation of the autolysis, samples were fixed in 3 % formaldehyde in 0.15 M Na-cacodylate-HCl buffer (pH 7.4 $\sim$ 1,350 mOsm). The fixation was performed at 0°C for 12 to 18 hours.

Following fixation the tissues were divided into two parts. Those intended for morphological studies were embedded in paraffin, cut, and stained with haematoxylin-eosin. The others intended for cytochemical demonstration of acid phosphatase (E. C. 3. 1. 3. 2.) were rinsed for 24 to 28 hours in 0.25 M sucrose in 0.01 M Na-cacodylate-HCl buffer (pH 7.4 $\sim$ 270 mOsm) at 0°C , and then rapidly frozen in a mixture of propane/propene (Gasol®, Esso) quenched by liquid nitrogen. The specimens were subsequently cut in 10 μm thick sections in a cryostat at -20°C and incubated in a modified Gomori medium (Barka & Andersson 1962), containing 0.25 M sucrose (Brunk & Eriksson 1972) for 45, 60, 90, or 120 minutes. The reaction product was visualized by immersion of the sections in a 1 % solution of $(\text{NH}_4)_2\text{S}$.

In the *in vivo*-study (paper V), the parietal decidua was obtained at various intervals

following intrauterine injection of 20 % saline solution either from aspiration abortion (in the eleventh to twelfth week of pregnancy), by hysterotomy (in the fifteenth to twentieth week of pregnancy), or by vacuum aspiration immediately after the abortion (in the fifteenth to twentieth week of pregnancy). In the hysterotomy cases, the saline solution was injected either by the extra-amniotic or the intra-amniotic route; in the vacuum aspiration cases, only the extra-amniotic method was used. Vacuum aspiration was performed either 15 minutes or 60 minutes following injection, or immediately after the abortion, which occurred 25 to 48 hours following the injection. The aspirated material was collected in a glass bottle kept on ice and containing 3 % formaldehyde in 0.15 M Na-cacodylate-HCl buffer (pH 7.4 $\sim$ 1,350 mOsm). The fixation was performed at $0 - 4^{\circ}\text{C}$ for 12 to 18 hours. In the cases terminated by hysterotomy, the operation was performed 3 hours after the injection. The operation included evacuation of the uterus as well as curettage of the uterine wall. The decidual tissue obtained was immediately placed in the above fixative solution and kept at $0 - 4^{\circ}\text{C}$ for 12 to 18 hours.

Following fixation the tissues were divided into two parts, one for morphological studies, and the other for cytochemical demonstration of acid phosphatase; both procedures were performed as described above.

Untreated decidua obtained either by vacuum aspiration or by hysterotomy served as control.

RESULTS AND COMMENTS

This survey of the findings in the present study is confined to the observations concerning the mode of action of the hypertonic saline. Other observations, such as the objective findings occasionally made of intravascular injection of the hypertonic saline, are not included.

Intrauterine spread of extra-amniotically injected hypertonic saline (papers I and II)
20 % saline solution, radiographically visible by added contrast medium and injected by the extra-amniotic route, was found to spread around the amniotic sac, either surrounding the entire sac or part of it. Since the amount of the solution injected was fairly large, i.e. about 150 to 200 ml, the findings indicate that the solution separated the membranes, either the entire membranes or part of them, from the decidua lining the uterine wall.

By measuring the sodium concentration in amniotic fluid following extra-amniotic injection, it was found that the saline soon diffused through the fetal membranes into the amniotic fluid and raised its salinity. In none of 19 patients studied, however, did the salinity rise above 4.4 %. This salinity is relatively low, compared with the initial concentration reached after intra-amniotic injection, which is somewhat below 20 % (Weingold et al. 1965, Csapo 1966, Andersson & Turnbull 1968, Pathak 1968, Frigoletto & Pokoly 1971, Kerenyi 1971, Wong et al. 1972).

Since the abortifacient effect of both injection-methods is about the same, the observations

suggest one of the following two possibilities:

- (1) The concentration of the intra-amniotically injected saline is unnecessarily high.
- (2) A higher salinity of the amniotic fluid is necessary to induce abortion when the saline is injected intra-amniotically than when it is administered extra-amniotically.

Amniotic fluid salinity and abortifacient effect (paper II)

Replacement of amniotic fluid by 5 % saline solution injected intra-amniotically did not result in abortion in any of 5 patients studied. In contrast, extra-amniotic injection of 20 % saline solution induced abortion in 16 out of 19 patients in spite of the fact that the salinity of the amniotic fluid did not rise above 4.4 %. It thus seems clear that the induction of abortion by hypertonic saline requires a higher salinity of the amniotic fluid when the saline is injected intra-amniotically than that reached when it is given extra-amniotically. This indicates that hypertonic saline does not induce abortion by acting on tissues inside the amniotic sac or on the fetal portion of the placenta, but rather by acting on extra-amniotic tissues. The decidua, which lies outside the amniotic sac, is directly exposed to the saline injected extra-amniotically, and the first uterine tissue reached by the saline injected intra-amniotically when the solution has diffused through the fetal membranes. It is therefore possible that the decidua is the target for the action of the hypertonic saline.

Effect on the placenta of intra-amniotically and extra-amniotically injected hypertonic saline (paper III)

In placentae aborted after either intra-amniotic or extra-amniotic injection of 20 % saline solution, a narrow zone of necrotic villi, haemorrhage and fibrin deposit was found immediately beneath the membranes. The thickness of this zone varied between one tenth and one fifth of the total thickness of the placenta. The zone was sharply demarcated from the underlying villi, which were microscopically intact. After extra-amniotic injection, the zone of damaged villi was slightly thinner than that after intra-amniotic injection.

The layer of decidual cells underneath Nitabuch's membrane showed advanced degenerative alterations with pyknotic or disintegrating cell nuclei, and extensive vacuolization and dissolution of the cytoplasm. The changes in the decidua were of the same appearance whether the hypertonic saline was injected intra-amniotically or extra-amniotically.

In the myometrium no histological changes could be observed.

Following intra-amniotic injection, the fetal part of the placenta is directly exposed to the hypertonic saline, which explains the necrotic zone observed. The zone was rather narrow, probably due to dilution of the hypertonic saline in the intervillous space and to absorption through the placental venous system.

Following extra-amniotic injection, the hypertonic saline diffuses through the fetal membranes into the amniotic fluid. As already described, the intra-amniotic concentration reached is lower than that achieved after direct intra-amniotic injection, which may explain why the necrotic zone was somewhat thinner after this type of injection.

The histological changes of the placenta observed in the present study were thus confined to a fairly narrow zone of necrotic villi immediately beneath the membranes. In the major part of the placenta, the villi were of

normal appearance, although finer lesions, non-detectable in the light microscope, cannot be excluded. Electron microscopic (Wynn 1965) and immunofluorescent (Christie et al. 1966) studies, however, have not revealed any damage to the trophoblast in this part of the placenta after intra-amniotic injection. It therefore seems unlikely that hypertonic saline can eliminate a progesterone "block" by virtue of a necrotizing effect on the progesterone producing part of the placenta.

The decidual cell damage, in spite of the fact that the basal villi look microscopically normal, is suprising. If the saline concentration in the basal part of placenta is high enough to damage the decidual cells without injuring the basal villi, it may be attributed to a greater vulnerability of the decidual cells than of the trophoblast.

In vitro study of the stability of decidual and trophoblastic cells (paper IV)

The apparent differences in stability of the decidual and trophoblastic cells were studied by comparing their vulnerability during osmotic stress and autolysis in vitro. Special attention was given to the lysosomal membranes, since derangement of these membranes would result in diffusion of hydrolytic enzymes with subsequent autolysis. Diffusion of lysosomal enzymes can be indicated by cytochemical methods for demonstrating the lysosomal "marker" enzyme acid phosphatase. When the normal pattern of granular reaction product is altered to a diffuse type of "staining", it suggests leakage of the enzyme from its initial lysosomal site (Brunk & Ericsson 1972).

It was found that fixation under "proper" osmotic conditions resulted in well-preserved cell morphology and a mainly granular type of reaction, both in decidual and trophoblastic cells. On the other hand, after fixation under "improper" osmotic conditions, as well as following autolysis for one hour, the decidual

cells showed pronounced morphologic changes and signs of profuse enzyme diffusion. In the trophoblastic cells, however, only slight changes were observed, even after autolysis for up to 8 hours. The resistance of the trophoblastic lysosomal membranes to autolysis thus seems to be within the same range as that in many other types of cells investigated by comparable methods (Andersson 1965, Goldblatt et al. 1965, Ericsson et al. 1967).

These findings suggest that the decidua consists of an exceedingly fragile population of cells with lysosomal membranes less stable than those of trophoblastic cells and cells in general. It should be borne in mind, however, that the damage was induced under artificial conditions. Further elucidation of the role played by decidual cells in the interruption of pregnancy required investigation *in vivo*.

In vivo effect on the parietal decidua of intra-amniotically and extra-amniotically injected hypertonic saline (paper V)

The stability *in vivo* of parietal decidual cells, obtained by vacuum aspiration or hysterotomy at various intervals after intrauterine injection of 20 % saline solution was studied histologically and cytochemically. The hypertonic saline was administered extra-amniotically in the vacuum aspiration cases, and either extra-amniotically or intra-amniotically in the hysterotomy cases. Fifteen minutes following the injection decidual cells in part of the decidua showed distinct degenerative alterations and signs of leakage to the cell sap of the lysosomal enzyme acid phosphatase. The extent of these changes and the number of cells involved increased with the interval between injection and evacuation.

In the material obtained by hysterotomy the parietal decidua showed alterations of the same type and approximately the same magnitude whether the hypertonic saline was injected intra-amniotically or extra-amniotically.

Decidual cells from patients not treated with hypertonic saline showed well-preserved morphology and a generally distinct granular distribution of the enzyme reaction product.

The observation that hypertonic saline rapidly damaged decidual cells *in vivo* is in agreement with the findings in the *in vitro* study (paper IV), where decidual cells proved exceedingly sensitive to various kinds of noxious treatment.

The progress of the damage to the decidual cells throughout the abortion, in spite of the fact that most of the injected hypertonic saline disappears from the uterus rather soon (Andersson & Turnbull 1968), might be explained as follows. Diffusion of acid phosphatase from the lysosomes into the decidual cell sap was rapidly induced in part of the decidua. Since lysosomal enzymes, taken together, can break down most or all cellular constituents, the diffusion of these enzymes into the cell sap would result in cellular degradation including decomposition of the plasma membrane (de Duve & Wattiaux 1966). Following such degradation, lysosomal enzymes would leak into the surrounding tissue and destroy still undamaged cells (for a review see Allison 1968). Moreover, the induced uterine contractions, when strong enough, may labilize decidual lysosomal membranes by hypoxia (de Duve 1959) and ischaemia (Ericsson & Brunk 1973), and also mechanically. Hence, uterine contractions, initiated by cellular damage in part of the decidua, might set in motion a chain of reactions leading to the labilization of membranes of other decidual cells.

It has thus been clearly shown in the present study that hypertonic saline injected by either route damages the decidual cells. One might wonder whether such a damage has any significance in the induction of the uterine contractions that follow the injection. It has been found in several tissues that even mild trauma releases prostaglandins (for ref. see

Horton 1972). High concentrations of these compounds have been demonstrated in decidual tissue during labor (Karim & Devlin 1967). Both extra-amniotic (Wiqvist & Bygdeman 1970, Embrey & Hillier 1971, Wiqvist et al. 1972) and intra-amniotic (Bygdeman et al. 1971, Karim & Sharma 1971) administration of prostaglandins can induce abortion. It seems therefore probable that the damage to decidual cells by hypertonic saline releases prostaglandins, resulting in uterine contractions and finally abortion.

It may be argued that prostaglandins would also be released from trophoblastic cells damaged by the hypertonic saline. However, neither in human term placental tissue (Karim 1967) nor in human malignant trophoblastic cells in culture (Patillo et al. 1973) could any prostaglandin activity be detectable.

Changes in prostaglandin $F_{2\alpha}$ concentration in amniotic fluid following extra-amniotic injection of hypertonic saline (paper VI)

To test the hypothesis that intrauterine injection of hypertonic saline induces prostaglandin release, the concentration of prostaglandin $F_{2\alpha}$ in amniotic fluid before and after extra-amniotic injection of 20 % saline solution was determined in 3 women. In all of them the amniotic fluid concentration of prostaglandin $F_{2\alpha}$ was increased 24 hours after the injection and thereafter showed a further increase.

The results thus indicate that prostaglandin $F_{2\alpha}$ is released into the amniotic fluid following extra-amniotic injection of hypertonic saline. But it is not possible to decide from these findings whether the release of prostaglandin is the cause or the consequence of the increased uterine activity.

GENERAL DISCUSSION

The findings in the present study, together with those made by others, make it probable that *one* of the factors involved in the action of intrauterine hypertonic saline in therapeutic abortion is the release of prostaglandins from damaged decidual cells. Such a release inside the very target organ, from a layer of cells lining the uterine cavity, would be highly effective, as the prostaglandins would thus escape the rapid inactivation known to occur during their circulation, mainly in the lungs.

Several procedures can start myometrial activity during pregnancy, including rupture of the membranes, sweeping the membranes off the lower uterine segment, injection of hypertonic saline, hypotonic rivanol and other substances, and even insertion of a rubber boogie into the uterus. Do these different procedures trigger off different mechanisms, or do they have a common mode of action? The present findings suggest that there might be a common target for them all, namely the lysosomes within the decidual cells.

Before presenting the arguments for this hypothesis, it might be useful to recall some details about the lysosomes. The lysosomes are small bag-like organelles bounded by a semipermeable membrane and containing various lytic enzymes. The enzymes can break down all the major constituents of the living cell: proteins, carbohydrates, lipids, and nucleic acids. Normally, they play a major role in intracellular digestion by fusing with vacuoles containing material to be degraded. However, if they are released from the lysosomes into

the cell sap, autolysis will occur, with lysis of the cell itself eventually being followed by destruction of tissues outside the cell. Such a release of lysosomal enzymes might occur when the permeability of the lysosomal membrane is increased or when the membrane is damaged. (de Duve 1959, de Duve & Wattiaux 1966, Allison 1968.)

One of the lysosomal enzymes, acid phosphatase, is commonly used as a "marker" enzyme for lysosomes, because it can be easily visualized by cytochemical methods. The findings of the present study suggest that intrauterine injection of hypertonic saline induces the release of the "marker" enzyme from the decidual lysosomes. The question now arises: What effect does such leakage have? As already mentioned, lysosomal enzymes are capable of breaking down most or all cellular constituents. It is also known that membrane phospholipids contain prostaglandin precursors. It therefore seems probable that lysosomal enzymes, by cleaving prostaglandin precursors from membrane phospholipids, provide substrate for prostaglandin synthesis. This would imply the following hypothetical sequence of events: Intrauterine injection of hypertonic saline —> Damage to decidual lysosomal membranes —> Leakage of lysosomal enzymes into the cell sap —> Intracellular processes resulting in prostaglandin synthesis —> Prostaglandin release —> Uterine contractions. Strong uterine contractions, however, cause hypoxia and ischemia in the uterus, and both these conditions have a labilizing effect on

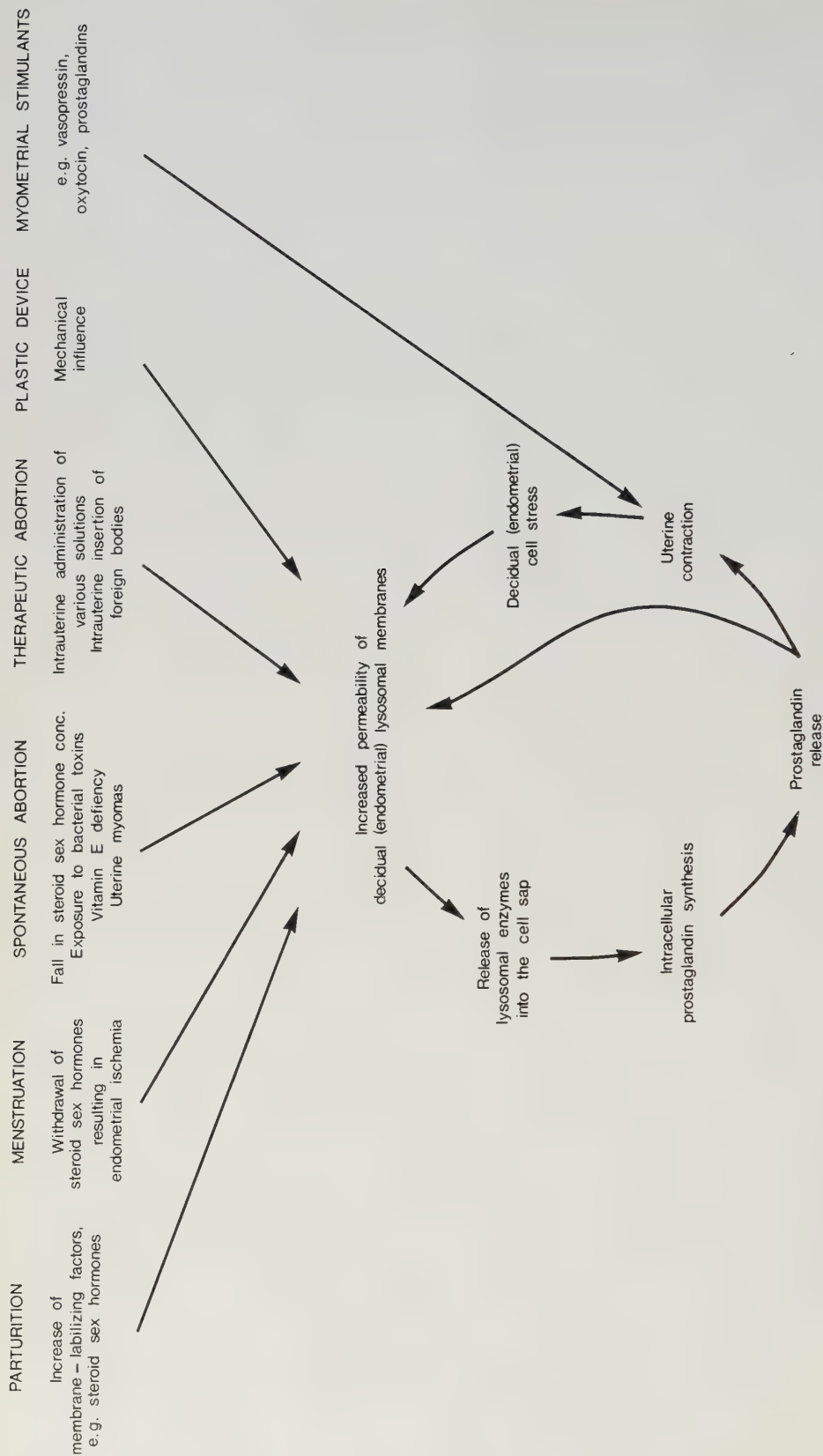


Fig. 1. Hypothetical scheme of reactions for the mechanism initiating contractions in the pregnant and non-pregnant uterus.

lysosomes, which means that they may induce a release of lysosomal enzymes. Therefore, this chain of reactions is better visualized as a cycle of reactions: contractions induced by hypertonic saline will in turn induce further contractions (Fig. 1). In the present study, the validity of this scheme of reactions is supported by (a) the extreme vulnerability of decidual cellular membranes, (b) the progress in decidual decomposition throughout abortion, and (c) the increase in prostaglandin $F_{2\alpha}$ of the amniotic fluid following the injection of hypertonic saline.

The findings of the present study thus suggest that the decidua consists of an exceedingly fragile population of cells with lysosomal membranes less stable than those in the trophoblastic cells and cells in general. Various factors influencing the stability of lysosomal membranes might therefore be of importance for the initiation not only of abortion but also of normal labor at term, and some of these factors are discussed below.

Studies *in vitro* have shown that progesterone labilizes lysosomes (de Duve et al. 1962, Bangham et al. 1965). Estradiol administered intravenously in physiological doses to rats labilizes lysosomes in preparations from the uterus and the preputial glands, but not in preparations from the lungs and the kidneys, suggesting an organ-specificity for this effect (Szego et al. 1971). Since progesterone and estrogen increase with the duration of pregnancy (for reviews see Beling 1971, and Solomon & Fuchs 1971) the labilizing action of these steroids will presumably also increase. Such a labilizing action would be highly effective, as the basal decidua lies close to the trophoblast, the main source of progesterone and estrogen during pregnancy. After a certain steroid concentration is reached at the end of pregnancy, leakage of enzymes from lysosomes into the cell sap might occur in the cells affected.

Once uterine contractions have started

other factors, such as spinal reflexes and the release of oxytocin and possibly vasopressin from the maternal as well as the fetal pituitary gland (Chard et al. 1971), will supervene and complete the act of parturition.

The decidua, like the non-pregnant endometrium, is shed after a certain period of time. It seems reasonable to suggest that the sequence of events in the non-pregnant uterus is induced in a way similar to that operating in the pregnant organ. For example, towards the end of the menstrual cycle, the lysosomes in endometrial cells increase in number (Henzl et al. 1972) and fragility (Bitensky & Cohen 1965). Some workers (for ref. see Henzl et al. 1972) believe that the withdrawal of the sex steroid hormones in the pre-menstrual phase produces endometrial ischemia due to contraction of the spiral arterioles. Since ischemia labilizes cellular membranes, a release of enzymes from the endometrial lysosomes could occur.

At the end of the luteal phase, the uterus becomes more sensitive to myometrial stimulants, especially vasopressin (for ref. see Coutinho 1968). The increase in myometrial response to pharmacological substances in parallel with the increase in fragility of endometrial lysosomes is suggestive. It is tempting to assume that the drug-induced contractions may influence endometrial lysosomes and by this action start the above-mentioned sequence of reactions. The effect on the *pregnant* uterus of myometrial stimulants, including prostaglandins, might possibly also be explained in a similar way. It may be added that prostaglandin $F_{2\alpha}$ has been found to increase lysosomal fragility by a direct action (Weiner & Kaley 1972).

Mechanical irritation of the secretory endometrium might also have a similar effect, since following insertion of a plastic device in humans, prelabor-like contractions appeared on the 19th day of the menstrual cycle instead of on the 25th day (thereby possibly disturbing

implantation of the fertilized ovum) (Bengtson & Moawad 1966). The observations in animal experiments that an intrauterine device increased the prostaglandin concentration in the endometrium in the proximity of the device, support this view (Spilman & Duby 1972).

As to the spontaneous abortion, certain women are liable to abort at the time when the hormone production of the corpus luteum is taken over by the trophoblast, a critical period when a fall in the sex steroid hormones can occur. It is also noteworthy that lyso-

somes are labilized by various bacterial toxins and by vitamin E deficiency (for ref. see Allison 1968).

Many factors are known to labilize lysosomes but few to stabilize them. This may explain why it is so easy to interrupt a pregnancy but so difficult to preserve it once uterine contractions have started. It is therefore of particular interest that aspirin has been shown not only to stabilize lysosomes (Miller & Smith 1966) but also to inhibit prostaglandin synthesis (Vane 1971).

GENERAL SUMMARY AND CONCLUSIONS

The present study of the mechanism by which hypertonic saline induces abortion has given the following results:

- (1) 20 % saline solution injected extra-amniotically (a) usually spread around the entire amniotic sac and thereby stripped the membranes off the decidua lining the uterine wall, and (b) diffused through the fetal membranes into the amniotic fluid and raised its sodium concentration.
- (2) Whether injected by the extra-amniotic or the intra-amniotic route, 20 % saline solution induced degenerative alterations in the decidual cells and leakage of the lysosomal enzyme acid phosphatase into the cell sap of these cells, while the major part of the trophoblastic cells remained microscopically intact. The extent of the decidual changes, and the number of cells involved, increased with the interval between the injection of the hypertonic saline and the evacuation of the uterine contents.
- (3) In contrast to trophoblastic cells, the decidual cells underwent rapid autolysis in vitro, and proved exceedingly sensitive to low effective osmotic pressure during fixation.

- (4) Extra-amniotic injection of 20 % saline solution was followed by the release of prostaglandin $F_{2\alpha}$ into the amniotic fluid.
- (5) The induction of abortion by hypertonic saline required a higher salinity of the amniotic fluid when the saline was injected intra-amniotically than that reached when it was given extra-amniotically.

The findings indicate that hypertonic saline does not induce abortion by acting on tissues within the amniotic sac or in the fetal portion of the placenta, but rather by acting on extra-amniotic tissues. The decidua, which lies outside the amniotic sac, is thought to be the target for the action of hypertonic saline. It is further assumed that the release of prostaglandin $F_{2\alpha}$ into the amniotic fluid is caused by damage to decidual cells, and that diffusion of lysosomal enzymes into the decidual cell sap might be the triggering mechanism for the release of prostaglandin. It would further seem probable that factors influencing the stability of lysosomal membranes are of importance in the initiation of labor and spontaneous abortion.

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